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Simon Jon McIlroy[1], Mads Albertsen[1], Eva Kammer Andresen[1], Aaron Marc Saunders[1], Rikke Kristiansen[1], Mikkel Stokholm-Bjerregaard[1,2], Kåre Lehmann Nielsen[1] & Per Halkjær Nielsen[1]

[1] Center for Microbial Communities, Aalborg University, Aalborg, Denmark
[2] Kruger A/S, Veolia Water Solutions and Technologies, Aalborg, Denmark

Introduction

The "*Candidatus* Competibacter"-lineage (henceforth Competibacter*)* exhibits a glycogen accumulating organism (GAO) phenotype, relying on aerobically stored glycogen to energize anaerobic carbon uptake and storage as polyhydroxyalkanoates (PHA).

This bi-phasic mode of life is important for Competibacter to survive in EBPR systems. However, as they do not contribute to phosphorus removal, but compete for organic resources with the PAOs, they theoretically reduce EBPR efficiency.

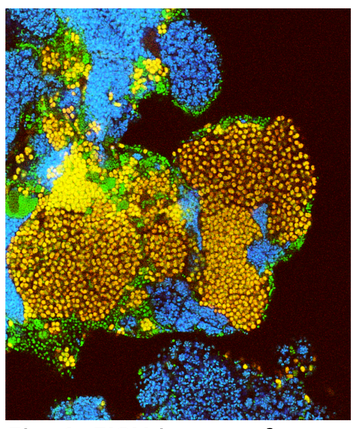


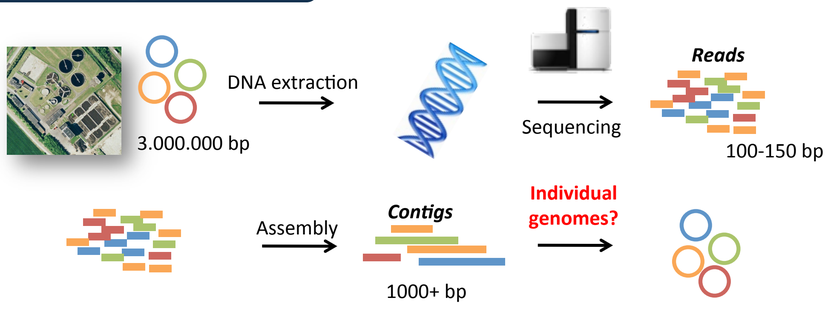
Fig. 1. FISH image of Competibacter (yellow)

Aim

To enhance our understanding of the Competibacter-lineage through the construction of genome-based-metabolic models.

Methods

Metagenome



Metagenome datasets generated from 2 lab-scale enrichments, seeded from a full-scale EBPR waste water treatment plant (WWTP).

Assembly

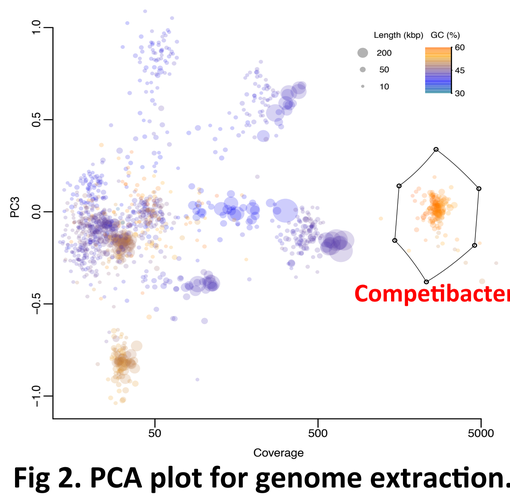


Fig 2. PCA plot for genome extraction.

De novo assembled genome fragments from 2 individual Competibacter strains extracted using tetranucleotide frequency, coverage, and GC content.

Annotation

Both genomes annotated using the 'MicroScope' pipeline (GenoScope) and curated for pathways relevant to metabolic model construction (**Fig. 5**).

Conclusions

This study is the first of its kind for organisms with the GAO phenotype and provides a solid foundation for future –omic based studies. The revealed potential for phenotypic diversity among these related *Competibacteraceae* species indicates potential niche partitioning and underlines the complex nature of interaction between the PAO and GAO populations in EBPR systems.

Results

Table 1. Metabolic comparison of pathways in sequenced GAO and PAO

	GAO$^{\varphi}$		PAO*				
	C. denitrificans	*C. odensis*	*Accumulibacter*	*T. elongata*	*T. australiensis*	*T. jenkinsii*	*T. japonica*
Central metabolism							
TCA cycle	+	+	+	+	+	+	+
Glyoxylate shunt	+	+	+	-	-	-	-
Pentose phosphate (oxidative)	-	-	+	+	+	+	+
Pentose phosphate (non-oxidative)	+	+	+	+	+	+	+
Glycolysis EMP pathway	+	+	+	+	+	+	+
Glycolysis ED pathway	+	-	-	-	-	-	-
Fermentation (glucose ➔ lactate)	+	-	-	+	-	-	+
Storage compound metabolism							
Glycogen synthesis	+	+	+	+	+	+	+
Trehalose synthesis	-	+	-	+	+	+	+
PHA synthesis	+	+	+	-	-	-	+
TAG synthesis	-	+	+	+	+	+	+
Polyphosphate synthesis	+	+	+	+	+	+	+
- Pit transporter	-	-	+	+	+	+	+
Nitrogen metabolism							
Nitrate reduction	+	+	-	+	+	+	+
Nitrate reduction to nitrogen (denitrification)	+	-	-	-	-	-	-
Nitrite reduction (respiratory)	+	-	+	+	+	+	+
Nitrite reduction to nitrogen	+	-	+	-	-	-	-
Nitrite reduction to ammonia (assimilatory)	-	+	+	-	-	-	+
Nitrogen fixation	-	+	+	-	-	-	-

φ This study; * Garcia-Martin *et al.*, (2006) *Nature Biotech.*; Kristiansen *et al.*, (2013) *ISME J.*

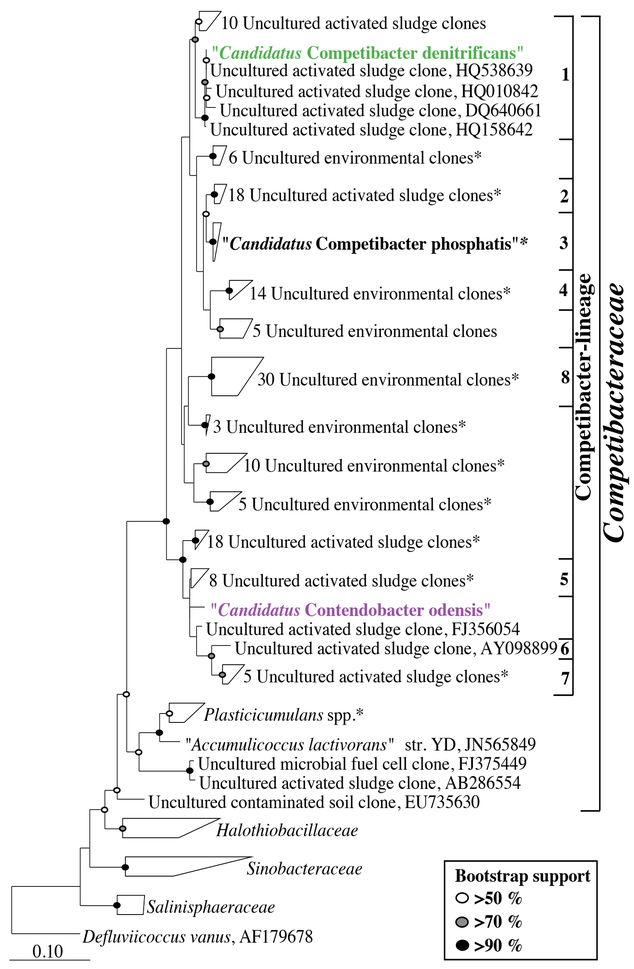


Fig 3. Phylogenetic tree for Competibacter-lineage sequences. Genome derived sequences are presented in colour. Phylogenetic analyses reveal genomes are members of Competibacter sub-groups 1 and 5. Diversity among the 'genus' Competibacter is high and thus the group better represents a family – *Competibacteraceae*.

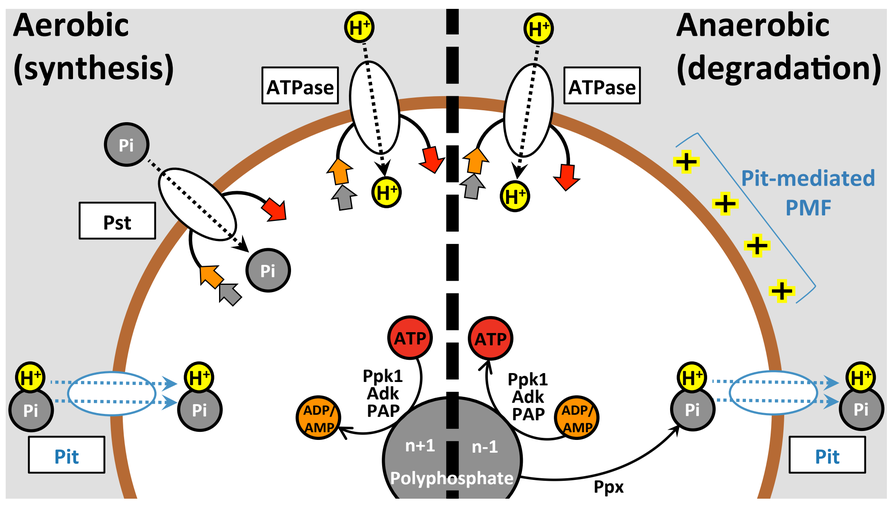


Fig. 4 Diagrammatic representation of P metabolism in PAO. Comparison of polyphosphate metabolism genes in available PAO and GAO genomes reveals the absence of the *pit* gene in the latter. The Pit phosphate transporter is theoretically involved in anaerobic proton motive force generation energising carbon uptake in PAO.

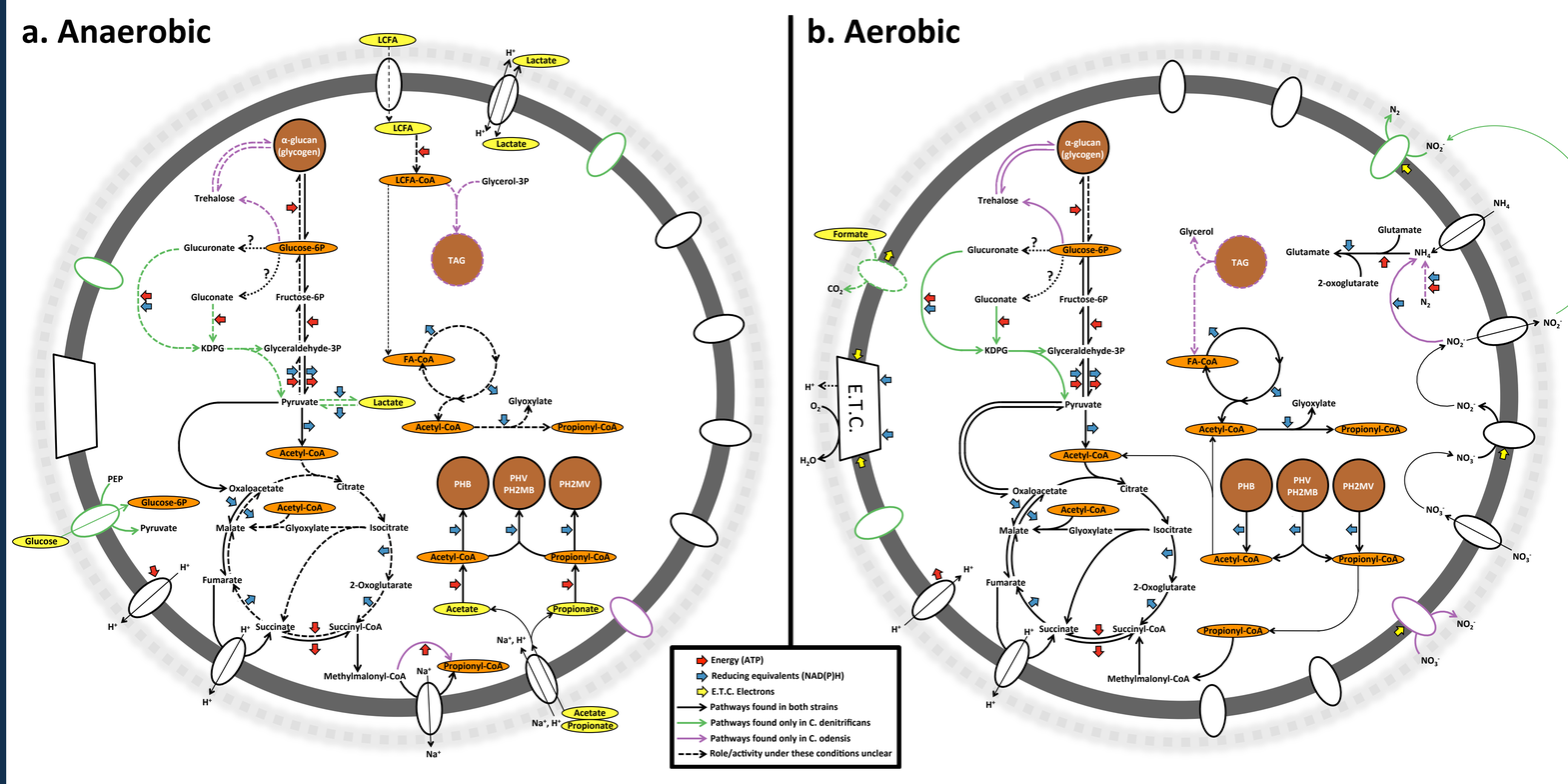


Fig 5. Metabolic model for Competibacter-lineage genomes in EBPR WWTPs a. in the absence of electron acceptors (anaerobic) and b. in the presence of the electron acceptors oxygen (aerobic) or nitrate. E.T.C = electron transport chain; PEP = phosphoenolpyruvate; KDPG = 2-dehydro-3-deoxy-D-gluconate-6-phosphate; TAG = triacylglycerols; PHB = polyhydroxybutyrate; PHV = polyhydroxyvalerate; PH2MB = polyhydroxy-2-methylbutyrate; PH2MV = polyhydroxy-2-methylvalerate. Both genomes contain genes for glycogen and PHA cycling and VFA metabolism. Differences between the genomes relate to storage polymers, nitrogen metabolism and glucose fermentation.

sjm@bio.aau.dk
www.cmc.aau.dk

KRÜGER

AALBORG UNIVERSITY

Environmental Biotechnology
Aalborg University